ISSN 0704-3716

Canadian Translation of Fisheries and Aquatic Sciences

No. 5424

The structure of "breeding marks" in the dentine of Odontoceti

G. A. Klevezal', L. I. Sukhovskaya, and A. Ya. Vilenkin

Original title: O strukture "metok razmnozheniya" v dentine Odontoceti

In: Zoologicheskii zhurnal 66(12): 1886-1891, 1987

Original language: Russian

Available from:
Canada Institute for Scientific and Technical Information
National Research Council
Ottawa, Ontario, Canada K1A 0S2

1988

15 typescript pages

Secretary of State / Secrétariat d'État

90-01522/

MULTILINGUAL SERVICES DIVISION – DIVISION DES SERVICES MULTILINGUES

TRANSLATION BUREAU BUREAU DES TRADUCTIONS

LIBRARY IDENTIFICATION – FICHE SIGNALÉTIQUE

Translated from - Traduction de	Into - En
Russian	English

Author - Auteur

G. A. Klevezal', L. I. Sukhovskaya, and A. Ya. Vilenkin

Title in English or French - Titre anglais ou français

THE STRUCTURE OF "BREEDING MARKS" IN THE DENTINE OF ODONTOCETI

Title in foreign language (Transliterate foreign characters)
Titre en langue étrangère (Transcrire en caractères romains)

O STRUKTURE "METOK RAZMNOZHENIYA" V DENTINE ODONTOCETI

Reference in foreign language (Name of book or publication) in full, transliterate foreign characters.
Référence en langue étrangère (Nom du livre ou publication), au complet, transcrire en caractères romains.

Zoologicheskii zhurnal

Reference in English or French - Référence en anglais ou français

Journal of zoology

Publisher - Éditeur	DATE OF PUBLICATION / DATE DE PUBLICATION			Page Numbers in original / Numéros des pages dans l'original
—	Year / Année	Volume	Issue No. / Numéro	1886–1891
Place of Publication / Lieu de publication				Number of typed pages / Nombre de pages dactylographiées
[USSR]	1987	66	12	13

Requesting Department / Ministère-Client: DFO

Translation Bureau No. / Notre dossier nº: 3416359

Branch or Division / Direction ou Division: Scientific Publications

Translator (Initials) / Traducteur (Initiales): MW

Person requesting / Demandé par: T. G. Smith

Your Number / Votre dossier nº: --

Date of Request / Date de la demande: July 14, 1988

UNEDITED TRANSLATION
For information only
TRADUCTION NON REVISÉE
Information seulement

OCT 28 1988

Canada

SEC 5-111 (84-10)

Secretary of State / Secrétariat d'État

MULTILINGUAL SERVICES DIVISION – DIVISION DES SERVICES MULTILINGUES

TRANSLATION BUREAU / BUREAU DES TRADUCTIONS

Client's No.–N° du client	Department – Ministère	Division/Branch – Division/Direction	City – Ville
–	DFO	Scientific Publications	Ottawa
Bureau No.–N° du bureau	**Language – Langue**	**Translator (Initials) – Traducteur (initiales)**	
3416359	Russian	MW	OCT 28 1988

Source: Zoologicheskii zhurnal (Journal of zoology), 1987, Vol. LXVI, No. 12, pp. 1886-1891 [USSR].

UDC 591.431.4 : 591.139 : 599.53

UNEDITED TRANSLATION
For information only
TRADUCTION NON REVISÉE
Information seulement

THE STRUCTURE OF "BREEDING MARKS" IN THE DENTINE OF ODONTOCETI

G. A. Klevezal', L. I. Sukhovskaya, and A. Ya. Vilenkin

"Breeding marks" - strongly contrasting layers in the dentine of female sperm whales - contain more calcium salts than the adjacent dentine and appear as two transparent bands, which are hyperchromatic when stained with hematoxylin, toluidine blue, and alcian blue. These two bands are separated by an opaque hypochromatic band that may be subdivided into 3 to 5 narrower and less contrasting strips. Dentine sclerotization was noted in the transparent bands of the strongly contrasting layers in females, while the presence of interglobular dentine and densely packed large globules of mineral salts were noted in the opaque band. The strongly contrasting layers of toothed whales is very similar in all these features to what has been described as the "calciotraumatic reaction" of dentine to the single administration of a number of substances, to parathyroidectomy, etc., whether the layers appear in their simple (in dolphins of the genus Stenella) or in their more complex (in sperm whales) forms. We conclude that complex strongly contrasting layers in female sperm whales are a dentine reaction to a single change in the concentration of a certain hormone during the breeding cycle - to the lower concentration of parathyroid hormone at parturition and at the beginning of lactation, for example. The strongly contrasting layers of male sperm whales is not complex and does not possess features resembling the "calciotraumatic reaction" of dentine.

During the past 25 years, the age of Odontoceti has been determined by counting the growth layers of dentine, which are alternating /1886*

*Translator's Note: Numbers in the right-hand margin refer to the corresponding page in the Russian text.

bands of varying optical density (see the survey by Perrin and Merrick, 1980). Transparant bands, which took on intensive color when stained, were particularly noticeable in certain growth layers. Such strongly contrasting layers were discovered in the dentine of teeth from sperm whales and from dolphins of the genus Stenella [Pike, 1966; Klevezal and Tormosov, 1971; Klevezal and Myrick,1984]. These strongly contrasting layers were regularly encountered in almost all adult females, with any given female displaying from one to ten strongly contrasting layers. Similar bands were also visible in males: in 10 percent of male sperm whales and in 30 percent of male Stenella, with no more than two or three in any individual male. An analysis of the correlation between (a) the frequency of occurrence of strongly contrasting layers in different growth layers and (b) the morphological and physiological characteristics of the females provided evidence for assuming that the formation of the strongly contrasting layers in females was related to the reproductive cycle[1] [Pike, 1966; Klevezal and Myrick, 1984]. This gave rise to the question: what are the strongly contrasting layers possessed by females and how similar is the structure of strongly contrasting layers in females to that in males?

In order to answer this question, we analyzed the structure of polished sections and of stained sections of teeth from 12 adult sperm, taken from the series of polished sections that was utilized in the work of Klevezal and Tormosov [1971].[2] These sections, with a thickness of

[1] Proceeding from the assumption of induced ovulation in sperm whales, Klevezal and Tormosov [1971] did not find it possible to relate the formation of strongly contrasting layers to the reproductive cycle. These authors recently reviewed their material in light of the latest information about the reproductive biology of sperm whales, and they came to the conclusion that there is most probably a relationship between the formation of strongly contrasting layers and the female reproductive cycle.

[2] We are deeply grateful to D. D. Tormosov for making it possible for us to analyze this material.

80 to 140 microns, were studied in both reflected and transmitted light. /1887
Cross sections with a thickness of 10 to 15 microns were obtained from the decalcified portions of the tooth and stained with Ehrlich's hematoxylin, alcian blue, toluidine blue, permanent green, and Heidenhain's azocarmine according to the manual of Romeis [1953] and that of Pirs [1962]. Since the material that we examined had been preserved for more than 20 years in a dry state, it was necessary to determine whether there was a difference in the action of these stains on (a) freshly fixed tooth tissue and on (b) tooth tissue that had been preserved for a long time in the dry state. For this purpose we utilized sections of the decalcified canine teeth obtained from a State fur farm and freshly fixed in neutral Formalin.

Curves characterizing the optical density of the strongly contrasting layers were obtained with a registering MF-4 microphotometer. The relative calcium salt content in the strongly contrasting layers of female sperm whales was determined by the method of local X-ray spectral analysis. For this purpose, a bivariate chart of the calcium concentration distribution was constructed on the surface of the polished section. The intensity of the characteristic X-ray emission Ca K_{α} was measured on the points in a rectangular area of 50 X 1000 microns, the long side of which was situated perpendicular to the strongly contrasting layers. The distance between the points along the short axis of the area was 10 microns; between the points on the long axis, 5 microns; that is, the chart consisted of 1000 points. The dimensions of the region in which the recorded X-ray emission was excited at each point was in the order of 1 micron in depth and 1 micron along the surface of the polished section. This operation was carried out with a JXA-733 apparatus. A curve of measurement of the relative calcium content was constructed by averaging the chart data for every 40 microns on the long side of the measurement rectangle.

The naked eye sees the strongly contrasting layers of the female sperm whale as a rather wide transparent band, considerably more transparent than the elements of the dentine growth layers (Figure 1, A).

The strongly contrasting layers of males appear identical, but perhaps just slightly less contrasting. According to Sukhovskaya [1986], who studied the polished sections of teeth from 126 females and 14 males in reflected light at a magnification of 2 X 8, the strongly contrasting /1888
layers of females, which varied from 100 to 600 microns in width, consisted of bands of varying optical density, in the typical case, of two narrow transparent bands separated by a dense [less transparent] band; the strongly contrasting layers of males were a uniform transparent band. The even more complex subdivision of the strongly contrasting layers of females is visible under the microscope in transmitted light, when examining polished microsections (Figure 1, B) and stained sections.

It can be seen from a microsection that the portions of strongly contrasting layers facing the pulp cavity and facing towards the periphery of the tooth are narrow bands of transparent dentine, and that the central portion is opaque dentine, which may be divided into a number of stripes of different optical density (Figure 2, A and B). Large (up to 10 microns) and densely packed mineral globules with narrow interglobular interstices between them are commonly - but not always - visible in the central band of the strongly contrasting layer (Figure 1, B). Dentine canals, which are readily visible outside the strongly contrasting layer, are scarcely discernable, as if they disappear in the transparent bands of the strongly contrasting layer. Stained sections of these bands show that there are no changes in the quantity or character of dentine canals in the strongly contrasting layer. Consequently, it follows that there is dentine sclerotization - the deposition of mineral salts - non only around, but also within the dentine tubules, as a result

of which they become difficult to see in the polished sections (for dentine sclerotization, see Bhaskar, 1980). Neither the subdivision of the strongly contrasting layer nor interglobular dentine nor dentine sclerotization in the strongly contrasting layer were found during the analysis of microsections of male teeth.

The strongly contrasting layer of females appears similar when stained with hematoxylin, alcian blue, or toluidine blue: two narrow hyperchromatic bands with a hypochromatic band between them, the latter consisting of three to five strips that differ in the extent to which they are stained. When stained with permanent green and azocarmine, the strongly contrasting layer begins and ends in hypochromatically narrow bands, while its central portion appears hyperchromatic. The strongly contrasting layer of males does not show this subdivision when stained; it stains somewhat more intensively with hematoxylin and less intensively with permanent green than do the basic elements of the growth layers.

X-ray spectral analysis showed that the strongly contrasting layer of females contains more mineral salts than the dentine surrounding it (Figure 2, A). This result agrees with the data obtained earlier [Klevesal and Sukhovskaya, 1983], indicating that the more transparent bands of dentine in marine mammals are hypercalcified in comparison to /1889
the opaque band.

A microsection boiled in alkali (by Schmidt's method) in order to remove collagen and then stained with silver nitrate clearly showed large, closely packed but not fused mineral globules in the central band of the strongly contrasting layer of females, but no differences were found in the structure of the mineral component in the transparent side

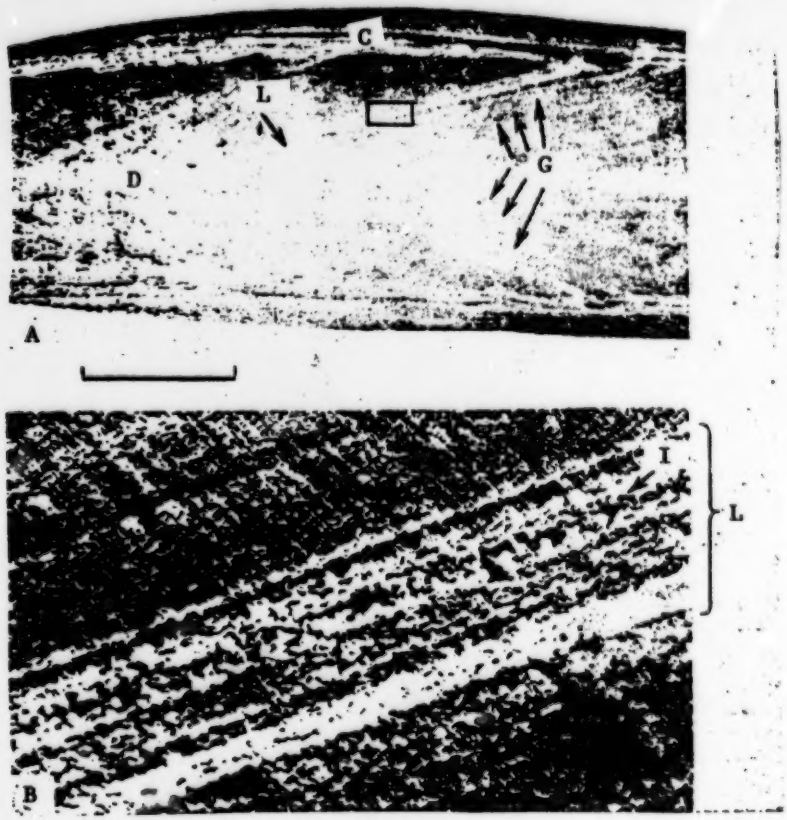

FIGURE 1

A - the polished section of a tooth from an adult female sperm whale in reflected light;

B - the area indicated by the box in the top photo, in transmitted light.

A - macrophotograph;
B - microscope, with objective 10 and eyepiece 3;
C - cement;
D - dentine;
G - growth layers
I - interglobular dentine;
L - strongly contrasting layer.

Scale: 1 cm.

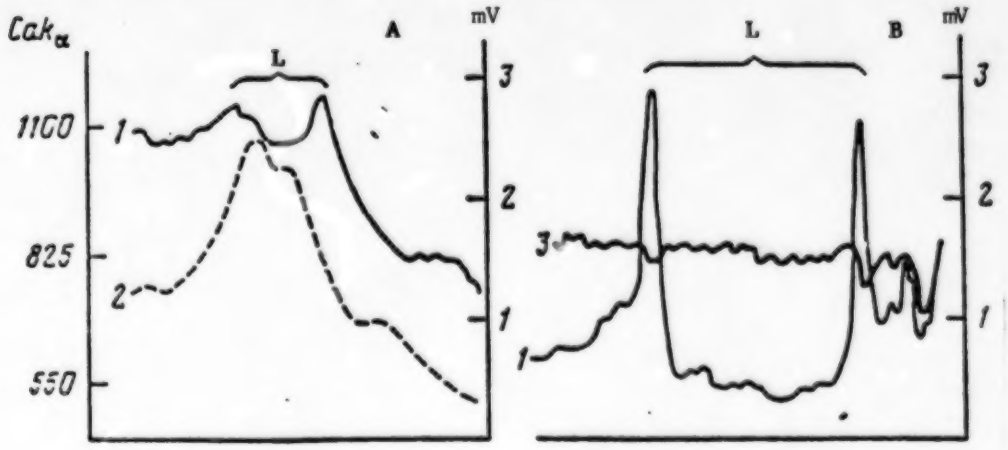

FIGURE 2

The characteristics of the strongly contrasting layers (L) of dentine from two female sperm whales (A, B):

1 - curves characterizing the optical density of the surface of a polished section of a portion of tooth. The peaks of the curve correspond to the transparent bands of dentine;

2 - variation in the relative calcium content in the area of the strongly contrasting layer;

3 - curve characterizing the optical density of the polished section after decalcification and staining with hematoxylin.

The scale on the right of the Y-axis indicates the current intensity, excited by the light beam passing through the polished section; the one on the left indicates the intensity, corrected for background, of the characteristic X-ray emission Ca K_α in standard units.

bands of the strongly contrasting layer or in the dentine adjacent to it.

Hematoxylin stained the elements of annual layers of dentine and cement in the canine teeth of an arctic fox in a similar manner both in sections obtained from freshly fixed material and in sections obtained from teeth that had been preserved for a long period in the dry state. In both sections of freshly fixed teeth from an arctic fox and in sections of sperm whale teeth that had been preserved in the dry state, hematoxylin, alcian blue, and toluidine blue stained intensively the very same elements of the growth layers, and these same elements were stained weakly by permanent green and azocarmine. This provides us with a basis for assuming that the stain features found for the strongly contrasting layer of a sperm whale are not a product produced by studying material preserved in the dry state.

When sections from the teeth of female sperm whales were stained with hematoxylin, alcian blue, and toluidine blue, the transparent bands of the strongly contrasting layer proved hyperchromatic (Figure 2, B); when stained with permanent green and azocarmine, these bands were hypochromatic. It is thought that hematoxylin intensively stains the hypercalcified portions of the dentine [Pizzolato and Lillie, 1968]. Permanent green and azocarmine re employed in histochemistry in order to detect total protein and collagen; alcian blue and toluidine blue, to detect acid mucopolysaccharides. In the event, it was demonstrated that toluidine blue enters into a reaction with calcium salts [Lillie, 1969]. Thus, we can conclude that the transparent bands of the strongly contrasting layer of females contain more clacium salts, less protein, and, possibly, more mucopolysaccharides than the opaque band of the layer.

The X-ray spectral analysis that we made did not disclose a reduced calcium salt content in the middle portion of the strongly contrasting layer, evidently because the majority of analyzed points in the central portion of the layer were located on the large mineral globules, and not on the narrow hypocalcified interglobular spaces.

When considering the strongly contrasting layer of whale teeth as a whole, we can see that this layer in dolphins of the genus Stenella is a hyperchromatic band, which is followed by a hypochromatic band (see Figure 1 in Klevezal and Myrick, 1984). The strongly contrasting layer of female sperm whales consists of a series of bands with different degrees of calcination and capable of being stained; it begins with a hypercalcined and hyperchromatic band, after which, in the simple case, are two, and in the complex case, a number of bands differing in degree of calcination and in staining capacity. If the strongly contrasting layer of female dolphins of the genus Stenella is indeed a reflection of parturition - the beginning of lactation in the formation of dentine [Klevezal and Myrick, 1984], then what is the formation of the complex strongly contrasting layer of sperm whales due to?

The literature on histology provides us with the concept of the "calciotraumatic reaction" of dentine [Seltzer and Bender, 1975]. This phenomenon was studied primarily on the incisors of laboratory mice and it refers to the formation of alternating bands of different degrees of calcination in dentine, which occurs when certain substances or foreign elements are introduced into the organism. The classic "calciotraumatic reaction" was described for the injection of fluoride [Osmanski and Yaeger, 1964; Yaeger, 1963] and refers to the formation of a narrow

hypercalcined band of dentine, which is followed by a hypocalcined band. Specimens stained with hematoxylin and alcian blue show this area of dentine as a hyperchromatic band followed by a hypochromatic band and (not /1890
always, but usually) one more hyperchromatic band. The hypocalcined (hypochromatic) band may be subdivided into two strips of normally calcinated dentine.

It is clear from this description that the strongly contrasting layer of dentine from Stenella females resembles the calciotraumatic reaction of dentine in its simple manifestation: hyper and hypochromatic bands; while the strongly contrasting layer of female sperm whales resembles that same reaction in its more complex variant, with two outside hyperchromatic bands and a subdivided central hypochromatic band. Dentine tubules are barely discernable in the hypercalcined band of the "calciotraumatic reaction" of dentine in polished sections, almost as if they had disappeared [Osmanski and Yaeger, 1964]; the size of the crystals in this band is similar to the size of the crystals in the nearby normal dentine, while in the hypomineralized band of the "calciotraumatic reaction" of dentine, the crystals are larger [Yaeger, 1963]. As stated previously, these same features were found in the strongly contrasting layer of female sperm whales.

In conclusion, the similarity of the structure of the strongly contrasting layer of dentine in female whales to the structure of dentine formed as a result of its "calciotraumatic reaction" is striking. This provides us with a basis for inferring that the complex strongly contrasting layer of the dentine of female whales is a result of the dentine's reaction, not to a series of changes in some physical parameters

or other of the individual, but to a single change. The "calciotraumatic reaction" of dentine has not only been described from the injection of a number of simple chemical substances, it has also been described from the injection of calciferol, vitamin D, from parathyroidectomy, and so on [Eisenmann and Yaeger, 1969]; in general, after parathyroidectomy in mice, gyperkalcicined dentine forms, consisting of alternating hyper, hypo, and hyperchromatic bands [Schour et al., 1937]. It thus becomes possible to explain the formation of the strongly contrasting layer in females, both female dolphins of the genus Stenella and female sperm whales, as the "calciotraumatic reaction" of dentine to a single, but very powerful change in the concentration of a particular hormone during the course of the reproductive cycle. This could be, for example, a reaction to a reduction in the concentration of parathyroid hormone, which occurs during the period of parturition [Pitkin et al., 1979]. The strongly contrasting layer of dentine in male sperm whales does not exhibit any of the signs characteristic of the "calciotraumatic reaction" of dentine, and the causes of its formation remain unclear.

As a phenomenon, the "calciotraumatic reaction" of dentine is of special interest in studying the identifying structures of mammals. It demonstrates that a single event in the life of an individual may be recorded by dentine in the formation of a structure that consists of not one, but of a number of elements.

WORKS CITED

1. Klevezal, G. A., and L. I. Sukhovskaya: 1983. "The causes of differences in the optical density of the layers of dentine in the teeth of marine mammals," Zool. zh., Vol. 62, No. 9, pp. 1407-1416.

2. Klevezal, G. A., and D. D. Tormosov: 1971. "The detection of local groupings of sperm whales according to the nature of the layers in the dentine of their teeth," Issledovaniya morskikh mlekopitayushchikh [Marine mammal studies], Tr. AtlantNIRO, Vol. 39, pp. 35-43.

3. Lilli [Lillie], R.: 1969. Pathological technique and practical histochemistry. Moscow: Mir Publishing House; pp. 1-645.

4. Pirs [Pierce ?], E.: 1962. Histochemistry: theoretical and applied. Moscow: Foreign Literature Publishing House; pp. 1-962.

5. Sukhovskaya, L. I.: 1986. "An investigation of the structure of breeding 'marks' in the dentine of sperm whale teeth," in Tezisy dokl. IX Vses. soveshch. po izuch., okhrane i rats. ispol'zovaniyu morskikh mlekopitayushchikh [Report summaries: IX All-Union Conference on the study, conservation, and rational exploitation of marine mammals]. Arkhangel'sk; pp. 372-374.

6. Romeis [?], B.: 1953. Microscopic techniques. Moscow: Foreign Literature Publishing House; pp. 1-718.

7. to 17. are in English.

Institute of Biological Development,
USSR Academy of Sciences (Moscow), and
Institute of Solid State Physics,
USSR Academy of Sciences (Moscow)

Received: December 2, 1986

1. *Клевезаль Г. А., Суховская Л. И.*, 1983. О причинах различий в оптической плотности слоев дентина зубов морских млекопитающих//Зоол. ж., *62*, 9, 1407—1416.
2. *Клевезаль Г. А., Тормосов Д. Д.*, 1971. О выделении локальных группировок кашалотов по характеру слоев в дентине зубов//Исследования морских млекопитающих. Тр. АтлантНИРО. 39, 35—43.
3. *Лилли Р.*, 1969. Патологическая техника и практическая гистохимия. М.: Мир, 1—645.
4. *Пирс Э.*, 1962. Гистохимия. Теоретическая и прикладная. М.: Изд-во иностр. лит., 1—962.
5. *Суховская Л. И.*, 1986. Исследование структуры «меток» размножения в дентине зубов кашалота//Тезисы докл. IX Всес. совещ. по изуч., охране и рац. использованию морских млекопитающих. Архангельск, 372—374.
6. *Ромейс Б.*, 1953. Микроскопическая техника. М.: Изд-во иностр. лит., 1—718.
7. *Bhaskar S. H. (ed.)*, 1980. Orban's oral histology and embryology. Toronto, London: The C. V. Mosby Co. St. Louis, 1—482.
8. *Eisenmann D. R., Yaeger J. A.*, 1969. Alterations in the formation of rat dentine and enamel induced by various ions//Arch. Oral Biol., *14*, 9, 1045—1064.
9. *Klevezal G. A., Myrick A. C.*, 1984. Marks in tooth dentine of female dolphins (genus *Stenella*) as indicators of parturition//J. Mammal., *65*, 1, 103—110.
10. *Osmanski P. C., Yaeger J. A.*, 1964. Microradiography of rat incisor dentin during the development of the response to injected fluoride//Anat. Rec., *148*, 3, 467—475.
11. *Perrin W. F., Myrick A. C. (eds.)*, 1980. Age determination of toothed whales and sirenians//Rep. Int. Whaling Commn. Spec., 3, 1—229.
12. *Pike G.*, 1966. Progress report on the study of sperm whales from British Columbia. Background paper//Spec. Sci. Meeting Int. Whaling Commn. Honolulu, 1—9.
13. *Pitkin R. M., Raynolds W. A., Williams G. A., Hargin G. K.*, 1979. Calcium metabolism during pregnancy: a longitudinal study//Amer. J. Obstet. Gynecol., *133*, 781—787.
14. *Pizzolato P., Lillie R. D.*, 1968. The impregnation of bone and pathologic calcification by metal salts and their recognition by unoxidized hematoxylin//Histochemie, *16*, 4, 333—338.
15. *Schour I., Chandeler S. B., Tweedy W. R.*, 1937. Changes in the teeth following parathyroidectomy. I. The effects of different periods of survival, fasting, and repeated pregnancies and lactations on the incisor of the rat//Amer. J. Pathol., *13*, 6, 945—970.
16. *Seltzer S., Bender J. B.*, 1975. The dental pulp. Biological considerations in dental procedures. Philadel., Toronto: J. B. Lippincott Co., 1—356.
17. *Yaeger J. A.*, 1963. Microscopy of the response of rodent dentin to injected fluoride// Anat. Rec., *145*, 2, 139—147.

STRUCTURE OF «BREEDING MARKS» IN ODONTOCETI DENTIN

G. A. KLEVEZAL', L. I. SUKHOVSKAYA, A. Ya. VILENKIN

Institute of Developmental Biology, USSR Academy of Sciences (Moscow),
Institute of Solid-State Physics, USSR Academy of Sciences (Moscow)

Summary

«Breeding marks», strongly contrasting layers in the dentin of sperm whale females, are hypercalcified against the adjacent dentin and consist of two transparent bands, hyperchromatic when stained with hematoxylin, toluidine blue and alcian blue, separated by an opaque hypochromatic band. The latter can be subdivided into 3—5 narrower and less contrasting layers. Sclerotization of dentin was noted in the transparent bands of strongly contrasting layers in females and the opaque band was packed with large mineral globules and with interglobular dentin. By all these features strongly contrasting layers of toothed whales are very much similar to the described in literature «calciotraumatic reaction» of dentin after a single administration of some substances, parathyroidectomy etc, in its simple (*Stenella*) or more complex (sperm whale) manifestation. Complex strongly contrasting layers in sperm whale females are concluded to be a dentin reaction after a single change in the concentration of a certain hormone during the breeding cycle, for example, due to lower concentration of parathyroid hormone at parturition and at the beginning of lactation. Pattern of strongly contrasting layers in sperm whale males is simple and does not resemble the «calciotraumatic reaction» of dentin.